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**A METHOD FOR PRODUCTION OF RED FOOD GRADE DYE**

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A METHOD FOR PRODUCTION OF RED FOOD GRADE DYE

Abstract/Preface

Field of application: Food industry. Substance of invention: Plant raw material is reduced to fine particles, sterilized, fermented with enzymes at joint cultivation of citric-acid fermentation microorganisms of *Aspergillus* and *Trishoderma genera*, then filtered and concentrated. 3 claims.

References/Prototypes

1. Russian Patent No. 2001073, IPC C 09 B 61/00, 1993/2

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This invention pertains to the isolation of red natural coloring agents from plant raw materials with microfungi; the invention may be applied to food industry.

There is a known method for red food grade dye production, which includes the reduction of plant raw material to fine particles, its sterilization, and fermentation with the enzymes of citric-acid fermentation microorganisms, including those of *Aspergillus* genus; this is followed by filtration and concentration.

The limitations of this method include the necessity to introduce food grade carbohydrates into substrate, incomplete assimilation of cellulose

—<sup>1</sup> Numbers in the margin indicate pagination in the foreign text.

that prevents deep extraction of the dye, and also low accumulation of citric acid that deteriorates color fastness.

The purpose of this invention is to simplify the composition of substrate, and also to increase the yield of coloring agents as a result of higher accumulation of citric acid at complete assimilation of cellulose.

With our proposed method for production of red food grade dye is realized through reduction of plant raw material to fine particles, its sterilization, and fermentation with the enzymes of citric-acid fermentation microorganisms of *Aspergillus* genus which is followed by filtration and concentration (as per the invention); additional fermentation is realized by the enzymes of microorganisms of *Trichoderma* genus at their joint cultivation with those of *Aspergillus*.

Thus due to formation of food grade carbohydrates in the process of cultivation it is possible to eliminate the necessity for their introduction into substrate and also to reduce the content of cellulose in culture fluid; this will increase the yield of dye and also improve its color fastness due to higher accumulation of citric acid.

It is preferable to use the fungi of *Trichoderma Koningi* or *Trichoderma longibranchiatum Rifai* species: based on their symbiosis with the fungi of *Aspergillus* genus it is possible to reach a more complete assimilation of poly- and oligosaccharides, reduce the content of cellulose in culture fluid, and also create the most favorable conditions for

accumulation of citric acid as a metabolite of the vital activity of *Aspergillus* fungi.

Further, it is preferable to realize fermentation using a method of deep cultivation.

In doing so, it is possible to improve productivity, and also to reduce the period of time required for substrate processing in parallel with improvement of intensity and uniformity of fermentation of the substrate.

The method is realized as follows:

Reduce a red-colored plant raw material to fine particles, thin the material with water (if required), then sterilize and inoculate with an inoculum of citric-acid fermentation microfungi of *Aspergillus* genus, preferably of *Trichoderma koningi* or *Trichoderma Rifai* species; thereafter cultivate the microorganisms jointly, preferably using a deep cultivation method. During the process of cultivation the substrate is subjected to the action of the microorganisms cultivated: this causes hydrolysis, assimilation of cellulose from the plant raw material applied, and also the accumulation of citric acid as a metabolite of the vital activity of *Aspergillus* fungi. Complete fermentation, then separate biomass from the culture fluid by filtration, and concentrate the fluid to obtain the target product. Characteristically, the content of cellulose in a cultural fluid depends on a species of *Trichoderma* microorganism applied and also on a method of cultivation: it may be either close to zero or equal to zero; on the other side, the accumulation of citric acid may be either similar to the prototype or somewhat higher than for the prototype.

Example 1. Reduce grape residue (Magarach rose-red grape variety) to fine particles, thin the raw material with water, then sterilize and inoculate with the microfungi of *Aspergillus citrullus* and *Trichoderma lecanii* species in the ratio of 2:1, thereafter cultivate them jointly using a deep cultivation method up to complete assimilation of all carbohydrates. Thereafter separate biomass from the culture fluid by tangential filtration, and concentrate the fluid with a reversed osmosis method up to 20 % content of dry substances. The resulting dye is not changing its color index over a six-month period of storage without adding of any stabilizers or preservatives.

Example 2. Wash and inspect common beet roots, then reduce them to fine particles, sterilize and inoculate with the microfungi of *Aspergillus niger* and *Trichoderma koningi* species in the ratio of 5:2; thereafter cultivate them jointly using a surface cultivation method up to complete assimilation of all carbohydrates. Thereafter separate biomass from the culture fluid, and concentrate the fluid in a rotary evaporator up to 65 % content of dry substances. The resulting dye does not change its color at its introduction into such confectionary products as caramels, ice cream, creams/custards, etc.

Example 3. Wash and inspect red cabbage, then reduce it to fine particles, sterilize and inoculate with the microfungi of *Aspergillus niger* and *Trichoderma koningi* species in the ratio of 3:1; thereafter cultivate them jointly using a deep cultivation method up to complete assimilation of all carbohydrates. Thereafter proceed with treatment as per Example 2 above. The result is the same as in Example 2.

Example 4. Reduce Aronia (*Aronia melanocarpa*) fruit residue to fine particles, thin the raw material with water, then sterilize and inoculate with the microfungi of *Aspergillus wentii* and *Trichoderma longibranchiatum Rifai* species in the ratio of 7:3, and then cultivate them jointly using a deep cultivation method up to complete assimilation of all carbohydrates. Thereafter separate biomass from the culture fluid using a filtering centrifuge, and concentrate the fluid using an ultrasonic spraying technology under vacuum up to a powdered state. The resulting dye when applied for a dry beverage formula is not changing its color index in the formula over one year.

In all of the above examples cellulose was assimilated completely; the dyes were completely transferred into culture fluids. As compared to the prototype with its up to 97 % yields of coloring agents, with our invention the yield was improved up to its maximal theoretically possible value. Further, the accumulation of citric acid with our invention was either on a level with the prototype or by 5 to 12 % higher. /4

Thus, with our invented method it is possible to simplify the composition of substrates, eliminate the necessity for application of food grade carbohydrates, and also to increase the yield of coloring agents and their color indices as a result of higher accumulation of citric acid at the complete assimilation of cellulose.

The Claims

1. A method for production of red food grade dye that includes the reduction of plant raw material to fine particles, its sterilization, fermentation with the enzymes of citric-acid fermentation microorganisms of *Aspergillus* genus, filtration and concentration; the method is characteristic of that additional fermentation is realized by the enzymes of microorganisms of *Trichoderma* genus at their joint cultivation with those of *Aspergillus*.

2. The method as per Claim 1 above characteristic of that as the microorganisms of *Trichoderma* genus is applied *Trichoderma koningi*.

3. The method as per Claim 1 above characteristic of that as the microorganisms of *Trichoderma* genus is applied *Trichoderma longibranchiatum Rifai*.

4. The method as per Claim 1 and Claim 3 above characteristic of that the fermentation is realized using a deep cultivation method.